HORIZONTAL GENE TRANSFER IN EVOLUTION: FACTS AND CHALLENGES

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ABSTRACT

The contribution of horizontal gene transfer to evolution has been controversial since it was suggested to be a force driving evolution in the microbial world. In this paper, I review the current standpoint on horizontal gene transfer in evolutionary thinking and discuss how important horizontal gene transfer is in evolution in the broad sense and particularly in prokaryotic evolution. I review recent literature asking firstly which processes are involved in the evolutionary success of transferred genes and secondly about the extent of horizontal gene transfer toward different evolutionary times. Moreover, I discuss the feasibility of reconstructing ancient phylogenetic relationships in the face of horizontal gene transfer. Finally, I discuss how horizontal gene transfer fits in the current neodarwinian evolutionary paradigm and conclude there is a need for a new evolutionary paradigm that includes horizontal gene transfer as well as other mechanisms in the explanation of evolution.

Recently, several calls have been put forward for a new evolutionary synthesis (Dean & Thornton 2007; Pigliucci 2007; Carroll 2008, Koonin 2009) that encompasses mechanisms other than mutation, natural selection and drift to explain evolutionary changes, as are developmental constraints and epigenetic modifications among others. Today it seems evident, from the studies discussed below, that horizontal (or lateral) gene transfer, whose direct visualization has been recently achieved (Babić et al. 2008), is an important force driving the evolution of Bacteria and Archaea, as well as that of unicellular eukaryotes, and should therefore also be considered as part of the structure of any evolutionary synthesis.

Horizontal gene transfer, "the non genealogical transmission of genetic material from one organism to another" (Goldenfeld & Woese 2007) is a source of new genes and functions to the recipient of the transferred genetic material. In this sense, it is a mechanism that permits the acquisition of evolutionary novelties. But these acquisitions are primarily non genealogical, questioning, in my opinion, the neodarwinian conception of a gradualist process driving the appearance of novel traits and functions.

In this review I will discuss how horizontal gene transfer fits in with current evolutionary thinking as well as the challenges that it proposes for the current evolutionary paradigm.

THE RISE OF HORIZONTAL GENE TRANSFER.

At the beginning, the founders of molecular phylogenetics used molecular information from different proteins and genes to reconstruct phylogenetical

relationships between organisms (Zuckerkandl & Pauling 1965). One of these molecular markers, the sequence of 16S RNA genes was proposed to be a good tool for reconstructing old phylogenetic relationships (Woese et al. 1990). It was soon realised however that different genes could lead to phylogenetical incongruence and conflictive phylogenies mainly in microorganisms (Hilario & Gogarten 1993, Gupta &Singh 1994; Golding &Gupta 1995; Whitehouse et al. 1998), by grouping together species or species groups that are split by other morphological, physiological or molecular markers.

On the other hand, mechanisms for transfer of genetic material between microorganisms were well known from the beginnings of molecular biology and molecular genetics research (Lederberg & Tatum 1946; Zinder & Lederberg 1952; Stocker et al. 1953) and the theoretical potential of cross-species gene transfer in evolution was soon proposed (Syvanen 1985).

On this background, the concept of horizontal gene transfer between organisms emerged at the beginning of the 1990´s as an alternative explanation for those conflictive phylogenetical events (Hilario & Gogarten 1993). Since then, new and abundant data has reinforced this idea especially with the rise of the genomic era, which has allowed the comparison of complete sets of genes between organisms.

Controversy is also associated to horizontal gene transfer. How important is it in evolution? Is it a challenge to the reconstruction of phylogenetic relationships between organisms? Is it possible to reconstruct a universal tree of life when the presence of horizontal gene transfer at some periods in the history of life is considered? What genes have been preferentially transferred and maintained thereafter? And one more important thing: How horizontal gene transfer challenges the traditional neodarwinian view of evolution as a gradual process of variation with descendence? I review below

the state of art in relation to these questions.

HOW IMPORTANT IS HORIZONTAL GENE TRANSFER IN EVOLUTION OF EARTH´S BIOTA?

Since the emergence of horizontal gene transfer as a way of explaining phylogenetic incongruence using different gene trees, a considerable number of studies have been published about genes that have been acquired by horizontal gene transfer (Gogarten et al. 2002; Lerat et al. 2005), both in Bacteria (Saunders et al. 1999; Ochman et al. 2000) and Archaea (Doolittle & Logsdon 1998; Faguy & Doolittle 1999), as well as in eukaryotes (Andersson 2005). These studies show that the transfer can occur not only among but also between domains in all possible directions: from Bacteria to Archaea (Rest & Mindell 2003), from Archaea to Bacteria (Gophna et al. 2004), from Archaea to Eukarya (Andersson et al. 2003), from Bacteria to Eukarya (Watkins & Gray 2006), from Eukarya to Bacteria (Guljamow et al. 2007) and even within Eukarya (Nedelcu et al. 2008). However, it is in Bacterial and Archaeal evolution where horizontal gene transfer has been more widely documented and accepted.

A recurrent question addressing the importance of horizontal gene transfer in evolution is how many genes in any given organism have been acquired by horizontal gene transfer. It is evident that in Bacteria and Archaea, even the transfer of a single or a few genes can give recipient organisms the opportunity to exert a new function, exploiting new ecological niches (Fournier & Gogarten 2008). However, its importance as an evolutionary mechanism can be limited if only a few horizontal gene transfer events have taken place in the history of the life.

Today, it is possible to address this question with respect to the evolution of Bacteria and Archaea using the abundant number of available completed genome sequences. However, in order to achieve this goal, it is necessary to previously identify what genes of an organism have been transferred. In this way, several criteria have been proposed and discussed (Ragan 2001; Ragan 2002; Lawrence & Ochman 2002) which permits this identification with more or less troubles: mainly bias in codon usage and different base composition in relation to others genes in the genome and phylogenetic incongruence.

Criteria based on codon usage bias and differential base composition have undergone several criticisms (Koski et al. 2001; Kuo & Ochman 2009). The foreign genes are ameliorated after many generations (Marri & Golding 2008) because both sets of genes, resident and transferred, support the same mutational biases once they are part of the same genome. In this way, these criteria could allow the identification of recent gene transfer events but are inefficient in detecting events that occurred a long time ago.

In relation to phylogenetic incongruence the problem is that it is difficult to differentiate in a a phylogenetic tree between horizontal gene transfer and gene loss (Ragan 2001; Ragan 2002; Lawrence & Ochman 2002; Snel et al. 2002; Zhaxybayeva et al. 2007). A careful comparison of the different phylogenetic methods employed along with the use of a test of compatibility between trees, can provide some clues about the process involved (Gogarten & Townsend 2005).

Despite these caveats, a lot of work has been carried out in the last few years to try to deal with the importance of horizontal gene transfer in Bacteria and Archaea evolution (Faguy & Doolittle 1999; Nelson et al. 1999; Ochman et al. 2000; Ochman 2001; Gogarten et al. 2002; Kurland et al. 2003; Philippe & Douady 2003; Pál et al.

2005a; Choi & Kim 2007; Koonin & Wolf 2008) , and the attained results are controversial depending on whether the emphasis is placed on gene transfer or gene loss (Kurland et al. 2003).

However, the emergent picture is that horizontal gene transfer plays a larger role in microbial evolution than previously thought.

Today it is estimated that between 1.6 and 32.6% of the genes of each microbial genome have been acquired by horizontal gene transfer (Koonin et al. 2001). Moreover, a recent study by Dagan et al. (2008) using a network analysis of shared genes in 181 sequenced prokaryotic genomes, shows that this percent increases dramatically to 81% $\pm$ 15% if the cumulative impact of horizontal gene transfer toward lineages is considered, which reinforces the importance of this mechanism in microbial evolution.

On the other hand, it has been assumed that the role of horizontal gene transfer in eukaryotic evolution is less important compared to its relevance in prokaryotic evolution. Evidently, the lower number of sequenced eukaryotic genomes compared to that of bacterial and archaeal genomes makes it difficult to estimate how many genes has been acquired by horizontal gene transfer in eukaryotes. However we are only starting to realise that horizontal gene transfer is not a negligible force in modulating eukaryotic genome evolution.

Presently, we already know that horizontal gene transfer is important in unicellular eukaryota evolution (Huang et al. 2004, Andersson 2005) and it is widely accepted that eukaryotic nuclear genomes contains several genes from microbial origin, that have been transferred from mitochondrial and plastids ancestors via ancient endosymbiotic events (Keeling & Palmer 2008; Lane & Archibald 2008).

But a recent study also highlights the role of horizontal gene transfer modulating

the evolution of other eukaryotic genomes in a different way: by acquisition of novel genes from living endosymbiontes (Hotopp et al. 2007).

In addition, it has been shown that the acquisition of foreign genes from bacteria and others eukaryotes is also important in fungi (Richards et al. 2006), and plant evolution (Martin et al. 1993, Huang & Gogarten 2088), and has contributed to the evolution of bdelloid rotifers (Gladyshev et al. 2008). On the other hand, horizontal gene transfer in eukaryotic evolution would be even more important if we consider animal and plant hybridization like a massive horizontal gene transfer event, as well as the proposed role of hybridization in evolution (Seehausen 2004).

In the following section I will focus the discussion on the impact of horizontal gene transfer in prokaryotic evolution.

HORIZONTAL GENE TRANSFER AND MICROBIAL EVOLUTION

The impact of horizontal gene transfer in microbial evolution (Bacteria and Archaea) is dependent on the number of genes that have been transferred to and successfully maintained in microbial genomes, but it is also dependent on the extent of the phenomena, both in evolutionary time framework (considering both recent and ancient events) and phylogenetic distance between organisms.

In this section I will review our current knowledge about the type of genes involved in transfer events and the way in which these genes are maintained post-transfer. This discussion can shed a light on two important questions concerning the impact of horizontal gene transfer in prokaryotic evolution. Has horizontal gene transfer been equally prevalent throughout evolution? Is horizontal gene transfer more prevalent between closely related organisms than between those than are distantly related?

Finally, I will review the impact that horizontal gene transfer has on the reconstruction of phylogenetic relationships between Bacteria and Archaea, as well as on the species concept in the prokaryotic world.

a) Gene transfer and the fate of transferred genes

Horizontal gene transfer results both from successful transfer of genetic material (mediated by process such as conjugation, transduction or transfection and ulterior recombination) and the survival of the transferred genetic material throughout the generations. The presence of certain physical barriers to transfer as well as different selective forces over the transferred genes may explain observed differences on the type of genes involved in horizontal gene transfer.

In 1999 Jain et al. proposed the complexity hypothesis (Jain et al, 1999) to explain the observed differences in horizontal gene transfer susceptibility between genes. This hypothesis proposes that the so-called informational genes (involved in DNA replication, transcription and translation, and whose products participate in multiple molecular interactions) are less prone to horizontal gene transfer than operational genes (involved in cell maintenance and whose products have few interactions with other molecules).

This hypothesis gained support from the characterisation by Bayesian inference of recently acquired genes in prokaryotic genomes (Nakamura et al. 2004), which has shown that the fraction of transferred genes is biased towards genes involved in DNA binding, pathogenicity and cell surface functions, all of them included among the functions of operational genes. This work also shows however that not all operational genes are participating equally in horizontal gene transfer events.

In addition, a recent study (Sorek et al. 2007) searched for genes that cannot be cloned in *Escherichia coli* in all of the completed bacterial and archaeal genomes. The authors found that, despite the fact that there aren´t any *a priori* barriers to cloning of any gene in *E. coli* (see below) some informational genes represent a substantial fraction of unclonable genes. However, there are some informational genes that can also be cloned.

Another recent study (Hao & Golding 2008), which used maximum likelihood models that incorporate rate variation to evaluate the contribution of gene insertions and deletion among 173 completed bacterial genomes, shows that according to the complexity hypothesis, informational genes are less prone to be involved in horizontal gene transfer than operational genes, but the difference between informational and operational genes is a small fraction of the overall observed variation.

Taking in all, these studies suggest that the functional split up of genes into those that are informational and those that are operational, isn't an absolute predictive tool for transferred genes.

A complementary approach to understand whether there are differences between genes, with regards to their involvement in transfer events, is to determine what processes are involved in post-transfer gene maintenance. It is accepted that the maintenance of a transferred gene is associated to positive selection (Gogarten et al. 2002, Pál et al. 2005b). In this way, genes having a useful function are preserved while useless genes are removed.

Several recent studies shed interesting results with regards to the maintenance of transferred genes (see review by Kuo & Ochman 2009). For example, it has been shown that the integration of a single transferred gene into regulatory interaction networks is very slow (Lercher & Pál 2008) in the case of genes providing the receptor with new

functions, and it is dependent on the number of partners for the gene product in the regulatory network, according to the complexity hypothesis. Moreover, products of these genes are integrated in the periphery of the corresponding regulatory network. On the other hand, transferred genes codifying for products with few partners are more easily deleted from genomes (Rocha 2008).

Recent work also shows that the transfer of complete operons enables the integration and maintenance processes allowing a rapid gain of function and facilitating the coordinate regulation of the new genes in the receptor (Price et al. 2008).

Taking in all, these results support that the involvement of their products in multiple molecular interactions (complexity) is a more important constraint to transfer and maintenance of genes in the prokaryotic world, than the functional class to which the transferred genes belong.

b) Incidence of Horizontal gene transfer over evolutionary time

The studies discussed above suggest that the evolutionary distance between organisms can be another important constraint with regards to transfer, because genes transferred between organisms separated long time ago where found to participate in very different regulatory networks. On the other hand, I have previously discussed that ancient transfer events are difficult to detect because of the amelioration process that affects the evolution of foreign genes in the receptor genome (Marri & Golding 2008, Almeida et al. 2008, Kuo & Ochman 2009).

Several studies suggest that, gene transfer could effectively be more frequent for short and intermediate evolutionary distances but uncommon between organisms that

are separated by large evolutionary time frames (Ochman et al 2000; Brugger et al. 2002; Nakamura et al. 2004; Ge et al. 2005; Choi & Kim 2007; Dagan et al. 2008). A recent study (Wagner & De la Chaux 2008) has analysed the evolution of 2091 insertion sequences in 438 completely sequenced prokaryotic genomes and found only 30 cases of presumed transfer events among distantly related clades. Twenty three of these events seemed to be ancient while only seven were recent.

However, instances of gene transfer between Archaea and Bacteria have been described (Rest & Mindell 2003, Gophna et al. 2004), which shows that horizontal gene transfer could affect the evolution in prokaryotic world along the different evolutionary times.

A recent study (Kanhere & Vingron 2009) compares the distance between orthologes and the intergenomic distances to try detect ancient transfer events in prokaryotic genomes. Authors found that 118 of the 171 gene transfer events were between Archaea and Bacteria and they correspond mainly to metabolic genes. Seventy four percent of these events were transfers from Bacteria to Archaea and the remaining twenty six percent were transfers from Archaea to Bacteria.

Only 53 genes were gene transfer events between bacteria phyla and corresponded mainly to genes involved in translation.

Despite the fact that this approach is limited to protein families that evolve at a constant rate, the study shows interesting trends within the interdomain gene transfer. It seems that the most transfers between Bacteria and Archaea have taken place in the Bacteria to Archaea direction.

On the other hand, studies by Zhaxybayeva et al. (Zhaxybayeva et al. 2006, Zhaxybayeva et al. 2009) show that the intra-phylum versus inter-phylum gene exchange is different among different bacteria lineages: in Cyanobacteria, intra-phylum

gene change seems to be more important than inter-phylum exchange (Zhaxibayeva et al. 2006). In Thermotogales however, and in particular in Firmicutes (Zhaxybayeva et al. 2009) the inter-phylum exchange is dominant over intra-phylum gene transfer. In addition, the proposed multiple gene exchange between Epsilon proteobacteria and Aquificales seems other example of inter-phylum exchange dominance (Boussau et al. 2008).

Taking in all, these studies suggest that despite the fact that gene transfer can be more frequent between closely related organisms, it may also take place between distantly related organisms, contributing to evolution of Archaea and Bacteria.

On the other hand, the fact that recent transfer events can be more easily detected adds a bias to the study of gene transfer in prokaryotic evolution confounding the real impact of ancient gene transfer events.

c) Prokaryotic phylogenetic relationships, bacterial species concept and horizontal gene transfer.

Reconstructing the phylogenetic relationships between Bacteria and Archaea, morphological characters are of limited use (Bohannon 2008) compared to metabolic and molecular markers. However, horizontal gene transfer challenge in many cases the correct reconstruction of these relationships, confounding the phylogenetic signal present in these markers. Some authors (Doolittle 1999; Martin 1999; Doolittle & Bapteste 2007) question whether it is possible to reconstruct an accurate phylogenetic tree for the microbial world at all, considering the existence of horizontal gene transfer events. Others support the idea that some core genes are never transferred (Wolf et al. 2002; Brown 2003) thus maintaining a true phylogenetic signal that enables the

reconstruction of a microbial phylogenetic tree. Finally, others (Kurland et al. 2003; Kurland 2005) consider that the existence of many barriers to gene transfer between organisms lowers the impact of horizontal gene transfer in phylogenetic reconstruction.

A recent study (Sorek et al. 2007) dealing with this topic searched in all the completed bacterial and archaeal genomes for genes that cannot be cloned in *Escherichia coli* as a proxy to study of barriers against horizontal gene transfer (see above). Their results suggest that there aren´t no absolute barriers to gene transfer because genes in all the families considered can be cloned in *E. col*i from at least one of the genomes.

In addition, the results of a network analysis of shared genes (Dagan et al. 2008) agree with the idea that horizontal gene transfer leaves no gene family untouched

Supporting the idea that horizontal gene transfer challenges reconstruction of phylogenetic relationships among prokaryotes, another study claims that less than 0.7% of the prokaryotic genes may be considered as core genes (Bapteste et al. 2008), making the construction of a phylogenetic tree unsustainable.

Recently, the Pan-genome concept (initially developed to determine how many genomes should be sequenced from any given bacterial species in order to get an accurate representation of the whole gene repertoire (Tettelin et al. 2005, Hogg et al. 2007, Tettelin et al. 2008) and to define the complete set of genes present in a prokaryotic group) has been applied to the study of the complete set of genes present in sequenced bacterial genomes (Lapierre & Gogarten 2009).

In this interesting study, the authors search for the presence of homologue genes in 573 completed genomes using BLAST and conclude that only 8% of the genes in a typical bacterial genome (~250 gene families in all genomes) are present in 99% of the sampled genomes and therefore can be considered to be core genes which are part of the

extended core genome or set of shared genes.

Lapierre & Gogarten identify two other gene categories: the so-called character genes (shared by a group of organisms) present in a subset of genomes (64% of the genes in a typical bacterial genome and 7900 gene families in all genomes) and the so-called accessory genes present in only one or in only a few of the genomes (28% of the genes in a bacterial genome and an infinite number of gene families in all genomes). Authors also suggest that character genes evolution is mainly based on mutation, gene duplication and horizontal gene transfer, while horizontal gene transfer and gene losses are involved in the evolutionary history of accessory genes.

The final conclusion of this study is that the bacterial "pan-genome" (the set of all genes present in bacteria) is of infinite size, demonstrating the plasticity of the genome evolution in prokaryotes.

Disregarding the fact that the methodology used can lead to underestimations or overestimations of particular gene categories, this study strongly underlines the impact that horizontal gene transfer has had on the evolutionary history of prokaryotes and provides us with important clues to understanding the evolution of prokaryotic genomes.

Bearing in mind the points discussed above, some authors support the point that it is impossible to reconstruct a tree of life, considering the pervasiveness of horizontal gene transfer in modulating Bacteria, Archaea and even Eukaryota genomes evolution. The metaphors of a web of life (Doolittle 1999) and a ring of life (Rivera & Lake 2004) have therefore been proposed as an alternative.

The web of life tries to represent phylogenetic relationships as a tree with many interbranch connections. On the other hand, the ring of life attempt to represent the three life domains as being connected in a single ring, considering that the eukaryotic

domain was originated from a hybridisation event between representatives from the Bacteria and Archaea domains (proposed by several authors but hotly debated (Embley & Martin 2006)) as well as the widespread gene exchange occurring between both domains.

Anyway, several tools have been proposed to deal with horizontal gene transfer in phylogenetic reconstruction, which encompass phylogenetic networks, supermatrices and supertrees (Huson & Bryant 2006; Galtier & Daubin 2008). The problem is that these tools aren´t free of criticisms (Rannala & Yang 2008). Even several authors propose that horizontal gene transfer may be used as a tool in phylogenetic reconstruction, providing with sinapomorphies that facilitate the reconstruction of problematic relationships (Huang & Gogarten 2006; Keeling & Palmer 2008).

Wagner and De la Chaux´ study discussed above (Wagner & De la Chaux 2008) which shows that horizontal gene transfer can be an infrequent event between distant clades, provides new arguments to those who defend the reliability of phylogenetic inferences in the microbial world. If Wagner and De la Chaux´results are true for other non-mobile DNAs, the chance of obtaining a reliable phylogenetic reconstruction in the microbial world will increase.

In addition, a new method for phylogeny reconstruction in prokaryotes based on genome organisation has been recently proposed (Merkl & Wiezer 2009) that seems robust to horizontal gene transfer events but may probably be regarded as limited to closely related organisms .

On the other hand, the recent study by Lapierre and Gogarten, discussed above (Lapierre & Gogarten 2009) could open the door to the dissection of the many evolutionary mechanisms converging in the evolution of bacterial lineages.

In this sense, Boucher and Bapteste (2009) very recent proposal of different

"closed lineages" (in which most of the evolutionary changes occurred by mutation) and "open lineages" (in which evolution was drive mainly by horizontal gene transfer) among prokaryotes is an interesting conceptual addition to the understanding of evolution in prokaryotes, in the meantime for developing and testing of new phylogenetic approaches.

Similar considerations have questioned the feasibility of microbial systematics (Bapteste & Boucher 2008) and have also challenged the species concept in the microbial world (Fraser et al. 2009) despite the fact that speciation processes can be established (Lawrence 2002).

According to some authors (McInerney et al. 2008) all of the traditional bacterial species can be considered to be populations of a single species taking in account the pervasive genetic exchange between bacteria. Others suggest that environmental cues may be used to define bacterial species (Cohan & Perry 2007, Ward et al. 2008). Other authors (Gevers et al. 2005) propose a sequential approach, using rRNA sequences to define prokaryotic Genera, as well as a multilocus sequence analysis (MLSA) to define species in the genera, and using different sets of genes for each genera or prokaryotic group.The most radical standpoints (Bapteste & Boucher 2008) propose a new systematic based on the redefinition of natural groups and evolutionary units considering the prevalence of horizontal gene transfer in the microbial world.

In this new frame, evolutionary units can be considered at different levels (composite evolutionary units), and the history of currently established entities can be split up into the different histories of their evolutionary units (transferred genes, vectors, etc). This new microbial systematic proposes the construction of a taxonomically interactive database which encompasses overlapping groups, as well as

the multiplication of names and taxa once biological units involved in the evolutionary process have been identified.

In this sense, the concept of open and closed lineages (Boucher & Bapteste 2009) discussed above can be very useful.

While waiting for new tools that will allow us to include horizontal gene transfer in phylogenetic reconstructions, the unanswered question still remains of whether horizontal gene transfer prevents us from using the tree paradigm in microbial evolution, as well as the species concept in the microbial world.

HORIZONTAL GENE TRANSFER IN CURRENT EVOLUTIONARY THINKING

Despite some recent attempts to integrate horizontal gene transfer in the neodarwinian paradigm of evolution (Arber 2008), horizontal gene transfer involves the exchange of genetic material between different organisms in a single generation.

Evidently, once transferred, natural selection can determine which genes spread throughout populations. But the acquisition of novel genes in a single generation, in my point of view, is far from the gradualist or slow change principle proposed by the neodarwinians (Dobzhansky 1937; Mayr 1993), and it has a certain Lamarckian taste (Goldenfeld & Woese 2007).

As has been suggested (Feder 2007), single-nucleotide modifications usually only modify existing genes and functions. The acquisition of new genes and functions requires other mechanisms. It is in this sense that horizontal gene transfer, along with gene or genome duplication, hybridisation and other mechanisms of gene acquisition

(Ohno 1970; Rivera & Lake 2004; Seehausen 2004; Feder 2007) can be considered as an evolutionary mechanism that challenges the neodarwinian paradigm (O´Malley & Boucher 2005, Koonin 2009).

I am aware that this is a disputed claim because many authors consider horizontal gene transfer as a process of rapid change and however gradualist (the question of what does the gradualism means in evolution deserves a more extended discussion and goes beyond the scope of this review). It is also true that not all authors agree with the relative importance of horizontal gene transfer in evolution, as has been discussed above.

But it is also true that authors are moving toward a continuous sense of acceptance (from strong rejecters and moderate rejecters to moderate accepters and strong supporters) in the light of new evidences (O´Malley & Boucher 2005), and this process is determining the shifting in the current paradigm.

It is in this sense that I think that horizontal gene transfer, along with the consideration of development as an internal evolutionary force, as proposed by EvoDevo (Gould 1977; Alberch 1982), epigenetic changes (Jablonka & Lamb 2005; Bird 2007) and other emerging concepts such as evolvability (Wagner 2008), etc, needs to be integrated in a new synthesis or paradigm, which will explain both eukaryotic and prokaryotic evolution. This new synthesis (without denying the role of natural selection, as well as other forces much-treasured by neodarwinians), needs to incorporate the emerging evolutionary knowledge (Dean & Thornton 2007; Pigliucci 2007; Carroll 2008, Koonin 2009) including mechanisms other than single-point mutations and gradual variability.

Only in this way will it be possible to come to a more robust evolutionary theory, which will be able to overcome the caveats of the neodarwinian theory or

Modern Synthesis, especially those that derive from "ad hoc" adaptationist explanations for the new knowledge.

Obviously, this proposal is not a new one -Darwin himself shows a pluralistic spirit about other forces involved in evolution (Darwin, 1859)- and different authors have proposed a similar integration of the different approaches like EvoDevo and comparative genomics among others. In this sense my position is that not all in evolution is black or white. Selection and neutral variation, phylogeny and development, gradualism and innovation, vertical and horizontal inheritance, every one of these is a piece of an intricate puzzle and it is thus necessary to piece them together to achieve a coherent understanding of evolution.

CONCLUSIONS

Horizontal gene transfer is an important force modulating evolution in the prokaryotic world and the evolution of particular eukaryotes. Although gene exchange is easier in closely related organisms, horizontal gene transfer occurred between both domains in the evolution of Archaea and Bacteria. However, it is a disputed point if horizontal gene transfer precludes the reconstruction of phylogenetic relationships in the microbial world. In any case, horizontal gene transfer is not a canonical or typical evolutionary mechanism. Thus, I agree with other authors in that there is a need for a new paradigm in evolution that includes horizontal gene transfer among other neodarwinian and non-neodarwinian mechanisms.

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REFERENCES

Alberch, P. 1982 Developmental constraints in evolutionary process. In *Evolution and Development* (ed. J. Bonner), pp 313-332. Berlín: Springer Verlag

Almeida, F. C.; Leszczyniecka, M.; Fisher, P. B.; DeSalle, R. 2008 Examining ancient inter-domain horizontal gene transfer. *Evol. Bioinform.* **4**, 109-119

Andersson, J.O. 2005 Lateral gene transfer in eukaryotes. *Cell. Mol. Life Sci.* **62**, 1182-1197 (DOI 10.1007/s00018-005-4539-z)

Andersson, J.O.; Sjögren, Å.M.; Davis, L.A.M.; Embley, T.M.& Roger, A.J. 2003 Phylogenetic analysis of Diplomonad genes reveal frequent lateral gene transfer affecting eukaryotes. *Curr. Biol*. ***13***, 94-104 (DOI 10.1016/50960-9822(03)00003-4)

Arber, W. 2008 Molecular mechanisms driving Darwinian evolution. *Math. Comput. Mode*l **47**,666 -674 (DOI 10.1016/j.mcm.2007.06.003)

Babić, A.; Lindner, A.B.; Vulić, M.; Stewart, E.J.& Radman, M. 2008 Direct visualization of horizontal gene transfer. *Science* **319**,1533-1536 (DOI 10.1126/Science.1153498)

Bapteste, E. & Boucher, Y. 2008 Lateral gene transfer challenges principles of microbial systematic. *Trends Microbiol*. **16**, 200-207 (DOI 10.1016/j.tim.2008.02.005)

Bapteste, E.; Susko, E.; Leigh, J.; Ruiz-Trillo, I.; Bucknam, J.& Doolittle, W.F. 2008 Alternative methods for concatenation of core genes indicate a lack of resolution in deep nodes of the prokaryotic phylogeny. *Mol. Biol. Evo*l. **25**, 83-91 (DOI 10.1093/mlbev/msm229)

Bird, A. 2007 Perceptions of Epigenetics. *Nature* **447**, 396-398 (DOI 10.1038/Nature05913)

Bohannon, J. 2008 Confusing kinships. *Science* **320**, 1031-1033 (DOI 10.1126/Science.320.5879.1031)

Boucher, Y.; Bapteste, E. 2009 Revisiting the concept of lineage in prokaryotes: a phylogenetic perspective. *Bioessays* **31**, 526-536 (DOI 10.1002/bies.200800216)

Boussau, B.; Guéguen, L. & Gouy, M. 2008 Accounting for horizontal gene transfers explains conflicting hypotheses regarding the position of aquificales in the phylogeny of Bacteria. *BMC Evolutionary Biol*. **8**:272 (DOI 10.1186/1471-2148-8-272)

Brown, J. 2003 Ancient horizontal gene transfer. *Nat. Rev.Genet*. **4**,121-132 (DOI 10.1038/nrg1000)

Brugger, K.; Redder, P.; She, Q.X.; Confalonieri, F.; Zivanovic, Y.& Garrett, R.A. 2002 Mobile elements in archaeal genomes. *FEMS Microbiol. Lett*. **206**, 131-141 (DOI 10.1111/j.1574-6968.2002.tb10999.x)

Carroll, S.B. 2008 Evo-Devo and expanding evolutionary synthesis. A genetic theory of morphological evolution. *Cell* **134**, 25-36 (DOI 10.1016/j.cell.2008.06.030)

Choi, I.G. & Kim, S.H. 2007 Global extent of horizontal gene transfer. *Proc. Natl. Acad. Sci.* **104**, 4489-4494 (DOI 10.1073/pnas.0611557104)

Cohan, F. M. & Perry, E. B. 2007 A systematics for discovering the fundamental units of Bacterial diversity. *Curr. Biol*. **17**, R373-R386 (DOI 10.1016/j.cub.2007.03.032)

Dagan, T.; Artzy-Randrup, Y. & Martin, W. 2008 Modular networks and cumulative impact of lateral transfer in prokaryote genome evolution. *Proc. Natl. Acad. Sci.* **105**,10039-10044 (DOI 10.1073/pnas.0800679105)

Darwin, C. 1859 *On the Origin of Species by means of Natural Selection or the Preservation of Favored Races in the Struggle for Life*, London: Murray

Dean, A.M. & Thornton, J.W. 2007 Mechanistic approaches to the study of evolution: The functional synthesis. *Nat. Rev. Genet*. **8**, 1604-1607 (DOI 10.1038/nrg2160)

Dobzhansky, T. 1937 *Genetics and the origin of species*, New York: Columbia University Press:.

Doolittle, W.F. 1999 Phylogenetic classification and the universal tree. *Science* **284**, 2124-2128 (DOI 10.1126/science.284.5423.2124)

Doolittle, W.F & Bapteste, E. 2007 Pattern pluralism and the tree of life hypothesis. *Proc. Natl. Acad. Sci.* **104**, 2043-2049 (DOI 10.1073/pnas.0610699104)

Doolittle, W.F. & Logsdon Jr. J.M. 1998 Do Archaea have a mixed heritage?. *Curr. Biol*. **8**, R209-R211 (DOI 10.1016/S0960-9822(98)70127-7)

Embley, T.M. & Martin, W. 2006 Eukaryotic evolution, changes and challenges. *Nature* **440**, 623-630 (DOI 10.1038/nature04546)

Faguy, D.M. & Doolittle, W.F. 1999 Lessons from the *Aeropyrum pernix* genome. *Curr.Biol.* **9**, R883-R886 (DOI 10.1016/S0960-9822(00)80074-3)

Feder, M.E. 2007 Evolvability of physiological and biochemical traits: evolutionary mechanisms including and beyond single-nucleotide mutation. *J. Exp. Biol*. **210**, 1653-

1660 (DOI 10.1242/jeb.02725)

Fournier, G. P.; Gogarten, J. P. 2008 Evolution of acetoclastic methanogenesis in *Methanosarcina* via horizontal gene transfer from cellulolytic *Clostridia*. *J. Bacteriol*. **190**: 1124-1127 (DOI 10.1128/JB.01382-07).

Fraser, C.; Alm, E. J.; Polz, M. F.; Spratt, B. G. & Hanage, W. P. 2009 The bacterial species challenge: Making sense of genetic and ecological diversity. *Science* **323**, 741-746 (DOI 10.1126/science.1159388)

Galtier, N.& Daubin, V. 2008 Dealing with incongruences in phylogenetic analyses. *Phil. Trans. R. Soc. B*. **363**, 4023-4029 (DOI 10.1098/rstb.2008.0144)

Ge, F.; Wang, L.S. & Kim, J. 2005 The cobweb of life revealed by genome scale estimates of horizontal gene transfer. *PLOS Biol*. **3**(10):e316 (DOI 10.1371/journal.pbio.0030316)

Gevers, D.; Cohan, F. M.; Lawrence, J. G.; Spratt, B. G.; Coenye, T.; Feil, E. J.; Stackebrandt, E.; Van de Peer, Y.; Vandamme, P.; Thompson, F. L. & Swings, J. 2005 Re-evaluating prokaryotic species. *Nat. Rev. Microbiol*. **3**, 733-739 (DOI 10.1038/nrmicro1236)

Gladyshev, E.A.; Meselson, M.& Arkhipova, I.R. 2008 Massive horizontal gene transfer in bdelloid rotifers. *Science* **320**, 1210-1213 (DOI 10.1126/science.1156407)

Gogarten, J.P.; Doolittle, W.F. & Lawrence, J.G. 2002 Prokaryotic evolution in light of gene transfer. *Mol. Biol. Evol*. **19**, 2226-2238.

Gogarten, J.P.& Townsend, J. P. 2005 Horizontal gene transfer, genome innovation and evolution. *Nat. Rev. Microbiol*. **3**, 679-687 (DOI 10.1038/nrmicro1204).

Goldenfeld, N.& Woese, C. 2007 Biology´s next revolution. *Nature* **445**, 369 (DOI 10.1038/445369a)

Golding, G.B.& Gupta, R.S. 1995 Protein based phylogenies support a chimeric origin

for eukaryotic genome. *Mol. Biol. Evol*. **12**, 1-6.

Gophna, U.; Charlebois, R.L.& Doolittle, W.F. 2004 Have archaeal genes contributed to bacterial virulence?. *Trends Microbiol* **12**, 213-219 (DOI 10.1016/j.tim.2004.03.002)

Gould, S.J. 1977 *Ontogeny and Phylogeny*, Cambridge, MA: Harvard Univ. Press.

Guljamow, A.; Jenke-Kodama, H.; Saumweber, H.; Quillardet, P.; Frangeul, L., Castets, A.M.; Bouchier, A. M.; Tandeau de Marsac, N.& Dittmann, E. 2007 Horizontal gene transfer of two cytoskeletal elements from a Eukaryote to a Cyanobacterium. *Curr. Biol*. **17**, R757-R759 (DOI 10.1016/j.cub.2007.06.063)

Gupta, R.S.& Singh, B. 1994 Phylogenetic analysis of 70 kD Heat- shock protein sequences suggest a chimeric origin for the eukaryotic cell nucleus. *Curr. Biol*. **4**,1104-1114 (DOI 10.1016/S0960-9822(00)00249-9)

Hao, W.& Golding, G.B. 2008 Uncovering rate variation of lateral gene transfer during bacterial genome evolution. *BMC Genomics* **9**:235 (DOI 10.1186/1471-2164-9-235)

Hilario, E.; Gogarten, J. P. 1993 Horizontal gene transfer of ATPase genes-the tree of life becomes a net of life. *Bio Systems* **31**: 111-119 (DOI 10.1016/0303-2647(93)90038-E)

Hogg, J. S.; Hu, F. Z.; Janto, B.; Boissy, R.; Hayes, J.; Keefe, R.; Post, J. C. & Ehrlich, G. D. 2007 Characterization and modeling of the *Haemophilus influenzae* core and supragenomes based on the complete genomic sequences of Rd and 12 clinical nontypeable strains. *Genome Biol.* 2007:**8** (DOI 10.1186/gb-2007-1188-1186-r1103)

Hotopp, J.C.D.; Clark, M.E.; Oliveira, D.C.S.G.; Foster, J.M.; Fischer, P.; Muñoz Torres, M.C.; Giebel, J. D.; Kumar, N.; Ishmael, N.; Wang, S.; Ingram, J., Nene, R. V.; Shepard, J.; Tomkins, J.; Richards, S.; Spiro, D. J.; Ghedin, E.; Slatko, B. E.; Tettelin, H. & Werren, J. H. 2007 Widespread lateral gene transfer from intracellular bacteria to multicellular eukaryotes. *Science* **317**, 1753-1756 (DOI 10.1126/science.1142490)

Huang, J. & Gogarten, J.P. 2006 Ancient horizontal gene transfer can benefit phylogenetic reconstruction. *Trends Genet*. **22**, 361-366 (DOI 10.1016/j.tig.2006.05.004)

Huang, J. & Gogarten, J. P. 2008 Concerted gene recruitment in early plant evolution *Genome Biol*. **9:**R109 (DOI 10.1186/gb-2008-9-7-r109)

Huang, J.; Mullapudi, N.; Lancto, C. A.; Scott, M.; Abrahamsen, M. S. & Kissinger, J. C. 2004 Phylogenomic evidence supports past endosymbiosis, intracellular and horizontal gene transfer in *Cryptosporidium parvum*. *Genome Biol*. **5**:R88 (DOI 10.1186/gb-2004-5-11-r88)

Huson, D.H. & Bryant, D. 2006 Application of phylogenetic networks in evolutionary studies. *Mol. Biol. Evol*. **23**, 254-267 (DOI 10.1093/molbev/msj030)

Jablonka, E. &Lamb, M.J. 2005 *Evolution in four dimensions: Genetic, epigenetic, behavioural, and symbolic variation in the history of life*, Cambridge, MA:MIT Press:.

Jain, R.; Rivera, M.C. & Lake, J.A. 1999 Horizontal gene transfer among genomes: The complexity hypothesis. *Proc. Natl. Acad. Sci.* **96**, 3801-3806.

Kanhere, A. & Vingron, M. 2009 Horizontal gene transfer in prokaryotes show differential preferences for metabolic and translational genes. *BMC Evol. Biol* **9**:9 (DOI 10.1186/1471-2148-9-9)

Keeling, P.J.& Palmer, J.D. 2008 Horizontal Gene Transfer in eukaryotic evolution. *Nat. Rev. Genet*. **9**, 605-618 (DOI 10.1038/nrg2386)

Koonin, E. V. 2009 Darwinian evolution in the light of genomics. *Nucleic Acids. Res*. **37**, 1011-1034 (DOI:10.1093/nar/gkp089)

Koonin, E.V.; Makarova, A.S. & Aravind, L. 2001 Horizontal gene transfer in Prokaryotes. Quantification and classification. *Annu. Rev. Microbiol*. **55**, 709-742.

Koonin, E.V. & Wolf, Y.I. 2008 Genomics of Bacteria and Archaea. The emerging

dynamic view of the prokaryotic world. *Nucleic Acid. Res*. **36**, 6688-6719 (DOI 10.1093/nar/gkn668)

Koski, L. B.; Morton, R. A. & Golding, G. B. 2001 Codon bias and base composition are poor indicators of horizontal gene transfer. *Mol. Biol. Evol*. **18**, 404-412

Kuo, C-H. & Ochman, H. 2009 The fate of new bacterial genes. *FEMS Microbial. Rev*. **33**, 38-43 (DOI 10.1111/j.1574-6976.2008.00140.x)

Kurland, C.G. 2005 What tangled Web: Barriers to rampant horizontal gene transfer. *Bioessays* **27**, 741-747 (DOI 10.1002/bies.20258)

Kurland,C.G.; Canback, B. & Berg, O.B. 2003 Horizontal gene transfer: A critical view. *Proc. Natl. Acad. Sci.* **100,** 9658-9662 (DOI 10.1073/pnas.1632870100)

Lane, C.E.& Archibald ,J.M. 2008 The eukaryotic tree of life: Endosymbiosis takes its TOL. *Trends Ecol. Evol*. **23**, 268-275 (DOI 10.1016/j.tree.2008.02.004)

Lapierre, P. & Gogarten, J. P. 2009 Estimating the size of the bacterial pan-genome. *Trends Genet*. **25**, 107-110 (DOI 10.1016/j.tig.2008.12.004)

Lawrence, J. G. 2002 Gene transfer in Bacteria: Speciation without species *Theor. Popul. Biol*. **61**, 449-460 (DOI 10.1006/tpbi.2002.1587)

Lawrence, J.G. & Ochman, H. 2002 Reconciling the many faces of lateral gene transfer. *Trends Microbiol*. **10**, 1-4 (DOI 10.1016/S0966-842X(01)02282-X).

Lederberg, J. & Tatum, E.L 1946 Gene recombination in *Escherichia coli*. *Nature* **158**, 558.

Lerat, E.; Daubin, V.; Ochman, H. & Moran, N.A. 2005 Evolutionary origins of genomic repertoires in Bacteria. *Plos Biol*. **3** (5): e130 (DOI 10.1371/journal.pbio.0030130)

Lercher, M. J. & Pál, C. 2008 Integration of Horizontally transferred genes into regulatory interaction networks takes many million years *Mol. Biol. Evol*. **25**, 559-567

(DOI 10.1093/molbev/msm283)

Marri, P.R. & Golding, G.B. 2008 Gene amelioration demonstrated: The journey of nascent genes in bacteria. *Genome* **5**1, 164-168 (DOI 10.1139/G07-105)

Martin, W. 1999 Mosaic bacterial chromosomes: A challenge en route to a tree of genomes. *Bioessays* **21**, 99-104

Martin, W.; Brinkmann, H.; Savonna, C. & Cerff, R. 1993 Evidence for a chimeric nature of nuclear genomes: Eubacterial origin of eukaryotic glyceraldehyde-3-phosphate dehydrogenase genes. *Proc. Natl Acad. Sci. USA* **90**, 8692-8696

Mayr, E. 1993 What was the evolutionary synthesis? *Trends. Ecol. Evol*. **8**, 31-33 (DOI 10.1016/0169-5347(93)90128-C)

McInerney, J.O.; Cotton, J.A. & Pisani, D. 2008 The prokaryotic tree of life: Past, present...and future? *Trends Ecol. Evol*. **23**, 276-281 (DOI 10.1016/j.tree.2008.01.008)

Merkl, R. & Wiezer, A. 2009 GO4genome: A prokaryotic phylogeny based on genome organization. *J. Mol. Biol*. **68**, 550-562 (DOI 10.1007/s00239-009-9233-6)

Nakamura, Y.; Itoh, T.; Matsuda, H. & Gojobori, T. 2004 Biased biological functions of horizontally transferred genes in prokaryotic genomes. *Nat. Genet*. **36**, 760-766 (DOI 10.1038/ng1381)

Nelson, K.E.; Clayton, R.A.; Gill, S.R.; Gwinn, M.L.; Dodson, R.J.; Haft, D.H.; Hickey, E.K.; Paterson, J.D.; Nelson, W.C.; Ketchum, K.A.; McDonald, L.; Utterback, T.R.; Malek, J.A.; Linher, K.D.; Garrett, M.M.; Stewart, A.M.; Cotton, M.D.; Pratt, M.S.; Philips, C.A.; Richarson, D.; Heidelberg, J.; Sutton, G.G.; Fleischmann, R.D.; Eilsen, J.A.; White, O.; Salzberg, S.L.; Smith, H.O.; Venter, J.C. & Frases, C.M. 1999 Evidence for lateral gene transfer between Archaea and Bacteria from genome sequence of *Thermotoga maritima*. *Nature* **399**, 323-329 (DOI 10.1038/20601)

Nedelcu, A.M.; Miles, I.H.; Fagir, A.M. & Karol, K. 2008 Adaptative eukaryote-to

eukaryote lateral gene transfer: stress-related genes of algal origin in the closest unicellular relatives of animals. *J. Evol. Biol*. **21**, 1852-1860 (DOI 10.1111/j.1420-9101.2008.01605.x)

Ochman, H. 2001 Lateral and oblique gene transfer. *Curr. Opin. Genet. Dev*. **11**, 616-619 (DOI 10.1016/S0959-437X(00)00243-4).

Ochman, H.; Lawrence, J.G. & Grolsman, E.A. 2000 Lateral gene transfer and the nature of bacterial innovation. *Nature* **405**, 299-304 (DOI 10.1038/35012500)

Ohno, S. 1970 *Evolution by gene and genome duplication*. Berlin: Springer.

O´Malley, M.A. & Boucher, Y. 2005 Paradigm change in evolutionary microbiology. *Stud. Hist. Phil. Biol. & Biomed. Sci*. 36, 183-208 (DOI 10.1016/j.shpsc.2004.12.002)

Pál, C.; Papp, B. & Lercher, M. J. 2005a Adaptative evolution of bacterial metabolic networks by horizontal gene transfer. *Nat. Genet*. **37**, 1372-1375 (DOI 10.1038/ng1686)

Pál, C.; Papp, B. & Lercher, M. J. 2005b Horizontal gene transfer depends on gene content of the host. *Bioinformatics* **21** Suppl 2, ii222-ii223 (DOI 10.1093/bioinformatics/bti1136)

Philippe, H. & Douady, C. J. 2003 Horizontal gene transfer and Phylogenetics. *Curr. Opin. Microbio*l. **6**, 498-505 (DOI 10.1016/j.mib.2003.09.008)

Pigliucci, M. 2007 Do we need an extended evolutionary synthesis?. *Evolution* **61**, 2743-2749 (DOI 10.1111/j.1558-5646.2007.00246.x)

Price, M. N.; Dehal, P. S. & Arkin, A. P. 2008 Horizontal gene transfer and the evolution of transcriptional regulation in *Escherichia coli Genome Biol*. **9**:R4 (DOI 10.1186/gb-2008-9-I-r4)

Ragan, M.A. 2001 Detection of lateral gene transfer among microbial genomes. *Curr. Opin. Genet. Dev*. **11**, 620-626 (DOI 10.1016/S0959-437X(00)00244-6)

Ragan, M.A. 2002 Reconciling the many faces of lateral gene transfer. *Trends. Microbiol*. **10**, 4 (DOI 10.1016/S0966-842X(00)02283-1)

Rannala, B. & Yang, Z. 2008 Phylogenetic inference using whole genomes. *Ann. Rev. Genomics Hum. Genet*. **9**, 217-231 (DOI 10.1146/annurev.genom.9.081307.164407)

Rest, J.S. & Mindell, D.P. 2003 Retroids in Archaea: Phylogeny and lateral origin. *Mol. Biol. Evol.* **20**, 1134-1142 (DOI 10.1093/molbev/msg135)

Richards, T.A.; Dacks, J.B.; Jenkinson, J.M.; Thornton, C.R. & Talbot, N.J. 2006 Evolution of filamentous plant pathogens: Gene exchange across eukaryotic kingdoms. *Curr. Biol*. **16**, 1857-1864 (DOI 10.1016/j.cub.2006.07.052)

Rivera, M.C.& Lake, J.A. 2004 The ring of life provides evidence for a genome fusion origin of eukaryotes. *Nature* **431**, 152-155 (DOI 10.1038/nature02848)

Rocha, E. P. C. 2008 Evolutionary patterns in prokaryotic genomes *Curr. Opin. Microbiol*. **11**, 454-460 (DOI 10.1016/j.mib.2008.09.007)

Saunders, N.J.; Hood, D.W. & Moxon, E.R. 1999 Bacterial evolution:Bacteria play pass the gene. *Curr. Biol*. **9**, R180-R183 (DOI 10.1016/S0960-9822(99)80108-0)

Seehausen, O. 2004 Hybridization and adaptative radiation. *Trends Ecol. Evol*. **1**9, 198-207 (DOI 10.1016/j.tree.2004.01.003)

Snel, B.; Bork, P. & Huynen, M.A. 2002 Genomes in flux: The evolution of Archaeal and Protobacterial gene content. *Genome Res*. **12**, 17-25 (DOI 10.1101/gr.176502)

Sorek, R.; Zhu, Y.; Creevey, C.J.; Francino, M.P.; Bork, P. & Rubin, E.M. 2007 Genome-wide experimental determination of barriers to horizontal gene transfer. *Science* **318**, 1449-1452 (DOI 10.1126/science.1147112)

Stocker, B.A.D.; Zinder, N.D. & Lederberg, J. 1953 Transduction of flagellar characters in *Salmonella*. *J. Gen. Microbiol*. **9**, 410.

Syvanen, M. 1985 Cross-species gene transfer: Implications for a new theory of

1 evolution. *J. Theor. Biol*. **112**,333-343.
2 Tettelin, H.; Masignani, V.; Cieslewicz, M. J.; Donati, C.; Medini, D.; Ward, N. L.;
3 Angiuoli, S. V.; Crabtree, J, Jones, A. L.; Durkin, A. S. et al. 2005 Genome analysis of
4 multiple pathogenic isolates of *Streptococus agalactiae*: implications for the microbial
5 "pan-genome". *Proc. Natl. Acad. Sci. USA* **102**, 13950-13955 (DOI
6 10.1073/pnas.0506758102)
7 Tettelin, H.; Riley, D.; Cattuto, C. & Medini, D. 2008 Comparative genomics: the
8 bacterial pan-genome. *Curr. Opin. Microbiol*. **12**, 472-477 (DOI
9 10.1016/j.mib.2008.09.006)
10 Wagner, A. 2008 Robustness and evolvability. A paradox resolved. *P. Roy. Soc. B.*
11 *Biol. Sci*. **275**, 91-100 (DOI 10.1098/rspb.2007.1137)
12 Wagner, A.& De la Chaux, N. 2008 Distant horizontal gene transfer is rare for multiple
13 families of prokaryotic insertion sequences. *Mol. Genet. Genomics* **280**, 397-408 (DOI
14 10.1007/s00438-008-0373-y)
15 Ward, D.M.; Cohan, F.M.; Bhaya, D.; Heidelberg, J.F., Kühl, M. & Grossman, A. 2008
16 Genomics, environmental genomics and the issue of microbial species. *Heredity* **100**,
17 207-219 (DOI 10.1038/sj.hdy.6801011)
18 Watkins, R.F.& Gray, M.W. 2006 The frequency of Eubacterium-to- Eukaryote lateral
19 gene transfer shows significant coss-taxa variation within Amoebozoa. *J. Mol. Evol*.
20 **63**, 801-814 (DOI 10.1007/s00239-006-0031-0)
21 Whitehouse, D.B.; Tomkins, J.; Lovegrove, J.U.; Hopkinson, D.A. & McMillan, W.O.
22 1998 A Phylogenetic approach to the identification of Phosphoglucomutase genes.
23 *Mol. Biol. Evol*. **15**, 456-462.
24 Woese, C.R.; Kandler, O. & Wheelis, M. 1990 Towards a natural system of organisms:
25 Proposal for the domains Archaea, Bacteria, and Eucarya. *Proc. Natl. Acad. Sci.USA*

87, 4576-4579.

Wolf, Y.I.; Rogozin, I.B.; Grishin, N.V. & Koonin, E.V. 2002 Genome trees and the tree of life. *Trends Genet*. **18**, 472-479 (DOI 10.1016/S0168-9525(02)02744-0)

Zhaxybayeva, O.; Gogarten, J. P.; Charlebois, R. L.; Doolittle, W. F. & Papke, R. T. 2006 Phylogenetic analyses of cyanobacterial genomes: Quantification of horizontal gene transfer events. *Genome Res*. **16**, 1099-1108 (DOI 10.1101/gr.5322306)

Zhaxybayeva, O.; Nesbo, C.L. & Doolittle, W.F. 2007 Systematic overestimation of gene gain through false diagnosis of gene absence. *Genome Biol*. **8**:402 (DOI 10.1186/gb-2007-8-2-402)

Zhaxybayeva, O.; Swithers, K. S.; Lapierre, P.; Fournier, G. P.; Bickhart, D. M.; DeBoy, R. T.; Nelson, K. E.; Nesbo, C. L.; Doolittle, W. F.; Gogarten, J. P. & Noll, K. M. 2009 On the chimeric nature, thermophilic origin, and phylogenetic placement of the Thermotogales. *Proc. Natl. Acad. Sci. USA* **106**: 5865-5870 (DOI 10.1073/pnas0901260106)

Zinder, N.D. & Lederberg, J. 1952 Genetic exchange in Salmonella. *J. Bacteriol*. **64**, 679-699.

Zuckerkandl, E. & Pauling, L. 1965 Molecules as documents of evolutionary history. *J. Theor. Biol*. **8**, 357-366